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analyzed by ddPCR. By this, we detected intra-tumor heterogeneity for HER co-amplifications. Here we present 2 tools based in MLPA that can identify the co-amplification level and the intra tumor heterogeneity of the 4 HER oncogenes, contributing to the precision medicine of breast cancer patients.

PS2-29 / Molecular cross-talk between ER and ID4 in breast cancer

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Inhibitor of differentiation (ID) 4, a member of the ID family, has been shown to act as a tumor suppressor and as an oncogene in breast cancer. Our group has investigated this apparent discordant information and has found evidence that ID4 acts as a tumor suppressor only in estrogen receptor ER+ tumors and as an oncogene only in ER- tumors. Here we focus on ID4's tumor suppressor role and further investigate why ID4 is aberrantly methylated exclusively in ER+ tumors. EZH2 is a histone methyltransferase involved in the tri-methylation of lysine 27 on histone 3 (H3K27me3) and also promotes DNA methylation via DNMT recruitment. In breast cancer EZH2 is overexpressed and downregulates the expression of tumor suppressor genes via increased promoter H3K27me3. Since ID4 is hyper-methylated in ER+ tumors and since EZH2 expression is induced by estradiol we hypothesize that estradiol induces ID4 methylation through EZH2. We performed siRNA (EZH2), immunofluorescence and chromatin immunoprecipitation (CHIP) experiments in MCF7 breast cancer cell lines. Our results show that EZH2 regulates ID4 expression as confirmed by siRNA experiments, that estrogen treatment increases EZH2 expression and CHIP experiments reveal that estrogen administration increases EZH2 and H3K27me3 marks on ID4 promoter. Taken together our results show for the first time that estradiol induces ID4 methylation trough EZH2 in breast cancer cell lines.

PS2-30 / Methods to monitor the relevance of M phase in the synthetic lethal potency of Polo-like Kinase 1 inhibitors

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Homologous recombination (HR) deficiency due to loss of BRCA function in cells leads to a propensity to the genesis of different types of cancer. Conversely, such HR deficiency is exploited to cause synthetic lethality (SL) or tumor-specific cell death in BRCA-deficient cancers. Such a synthetic lethality can be achieved by using drugs such as PARP inhibitors (PARPi) that prompt the accumulation of substrates for HR, e.g., DNA double-strand breaks (DSBs), which cannot be repaired in HR deficient cells. The trigger for SL in BRCA deficient cells treated with PARPi is intimately associated with acute DNA replication stress. In contrast, we have recently reported that BRCA1-deficient cells can be killed in a manner independent from such an S phase-associated stress. We found that inhibition of PLK1, an M-phase master kinase, causes SL in BRCA1 deficient models in a manner that does not augment parameters of DNA replication stress. Instead, BRCA1-deficient cells treated with PLK1i aggregate into multinucleated structures that suggest M phase's role in the SL triggered by PLKi. To get insight into such a DNA replication stress-independent SL mechanism, we will systematically monitor chromosome segregation, and other M phase parameters (multipolar mitoses, chromosome bridges, and lagging chromosomes resolution) will be discussed in depth during the presentation of results.

PS2-31 / Epigenetic modifications associated to breast cancer in metabolic syndrome-like disease mice models

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Metabolic syndrome (MS) is a proven risk factor for Breast Cancer (BCa). Previously, we found prominent epithelial lining in breast ducts of MS-like disease mice, obtained by chronically feeding animals with high fat diet (HFD). Our aim was to assess epigenetic alterations in breast ductal epithelium and tumors from HFD fed mice. H&E stains from breast tissues obtained from HFD fed Balb-c mice showed increased nuclei size and mitotic rate with presence of apoptotic bodies and nucleoli compared to control diet (CD) mice. Breast ductal epithelium from these mice were evaluated by immunohistochemistry (IHC) using antibodies against DNA methylation (5MC) and methylated histones (3MeH3-K4, -K9, -K27 and 2MeH3K36). We found no differences between groups by immunoreactive score. Nu/nu HFD or CD fed mice were inoculated with MDA-MB-231 cells. Xenografts showed no differences in methylated histones expression between groups, but HFD showed an increase of 5MC (IHC) and its enzyme DNMT1 (RT-qPCR) expressions. Analysis of multiple microarray datasets from patients (Oncomine) showed EZH2 and DNMT1 upregulated in BCa compared to normal breast tissue. Additionally, analysis of functional genomic datasets (UCSC Xena) revealed significantly increased (DNMT1, EZH2, SUV39H1, SUV39H2) or decreased (EZH1, SMYD1, SETD7) expression of methyltransferases in BCa tissue compared to normal tissue. We propose DNMT1 and EZH2 as potential biomarkers to further explore MS-associated BCa diagnosis.

PS2-32 / Epigenetics, bioelectricity and laterality of breast cancer

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In previous studies we found unexpectedly in patients that left-right (L-R) breast cancers (BC) differed in their methylation profiles (DM). We opened a new research line in which we hypothesize that, given the L-R environments of breast glands are non-identical: i. the bioelectric communication of the tumor with the L-R context differs,

and ii. epigenetics has a crucial role in these differences. Our results, so far, are promising. We found in-silico that the top genes with L-R DM were involved in development, embryogenesis, and neural differentiation. We confirmed the same processes, by developing a MDA-MB231-Nod Scid Gama xenograft model and compared L-R tumoral methylation patterns by RRBS. With focus on ion channels, we found that depolarizing channels were more methylated in R breast tumors. This suggested that R sided tumors had a more polarized state as compared to L tumors. We setup an in-vitro model to treat MDA-MB231 cells with L-R conditioned extracts from normal human mammary glands and measured Ca2+ and $\Delta\psi p$ with fluorescent probes. Cytometry assays confirmed bioelectric differences in the same direction: a more polarized state of right-treated cells. When deepening on epigenetic regulators, we found in-vitro a subtle increase of DNMT3 (de-novo methyltransferase) in left-treated cells, and confirmed it in-silico. Our studies support a non-explored epigenetic-bioelectric-laterality hypothesis for BC, which could serve as proof-of-principle for other bilateral tumors.

PS2-33 / Investigating the role of CBFB in breast cancer
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CBFβ is one of the top 17 most recurrently mutated genes in breast cancer. As a crucial transcriptional co-activator, CBFβ improves the DNA-binding affinity of RUNX proteins and therefore transcription of RUNX target genes. Since RUNX proteins have been previously shown to play context dependent roles in breast cancer and CBFβ is an essential regulator of these proteins, we questioned whether it has a phenotypic consequence in this disease setting. In silico analysis of TCGA data using cBioPortal highlighted how CBFβ undergoes varying alterations depending on the subtype of breast tumours. Interestingly in vivo experiments using a MMTV-PyMT;MMTV-Cre, Cbfβfl/fl mouse model of breast cancer, did not present any overt effects on tumorigenesis. This may be due to the mosaic nature of MMTV-Cre expression in PyMT driven tumours and as such we are testing whether Cbfβ is deleted in these tumours through western blotting alongside incorporating an RFP reporter gene for fluorescence imaging of tumours in vivo. Additionally, we have generated inducible-Cre tumour-derived cell lines (MMTV-PyMT;ROSA-CreERT2;Cbfβfl/fl) and conducted a range of biological assays to determine the effects of acutely removing CBFβ on tumorigenesis ex vivo. Preliminary results show reduced rates of growth in tumour cells lacking CBFβ. Together these results will provide an insight into the context dependent roles of CBFβ in different models of breast cancer.

PS2-34 / Analysis of RUNX-CBFβ as a relevant regulator of RSPO3 expression in breast cancer cells
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We have recently determined that R-spondin3 (RSPO3), a secreted protein that potentiates Wnt signaling pathway, is a key modulator of tumor progression and stem cell behavior in basal breast cancer. Previous reports suggested the potential involvement of the RUNX-CBFβ axis on RSPO3 expression in mammary tumor cells. These preliminary observations were confirmed by our results showing that small molecules able to inhibit CBFβ-RUNX interaction caused reduction of RSPO3 mRNA and protein levels in MDA-MB231 breast cancer cells. These treatments also induced inhibition of cell migration, ability that was recovered upon addition of recombinant RSPO3. To further explore the mechanisms underlying the control exerted by RUNX-CBFβ on RSPO3, we performed an in silico analysis of publicly available data from two RUNX1 CHIP-seq reports and an ATAC-seq study from human breast cell lines. We aligned the emerging data with the occurrences of the RUNX1 DNA-recognition-motif in the Rspo3 locus. This approach revealed a few putative RUNX1 binding sites. Among them, an intronic Rspo3 region that seems to be particularly active in triple negative (TN) breast cancer cells deserves special attention. In summary, our results show that RUNX-CBFβ transcriptional activity might affect TN mammary tumors by controlling RSPO3 expression levels. More experiments are being carried out to determine the mechanisms involved and the impact of this pathway on TN breast cancer behavior.

PS2-35 / RUNX2 overexpression generates endocrine resistance in human luminal breast cancer xenografts
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T47D and IBH6 cells that overexpress RUNX2 show high levels of FGFR2 and FGF2, supporting the hypothesis that FGF2 increases RUNX2 and, in turn, RUNX2 increases FGF2, maintaining a positive loop. However, in these models RUNX2 overexpression generates tumor resistance to FGFR inhibitor therapy and show a more aggressive phenotype compared with control tumors. T47D and IBH6 are luminal breast cancer cells that express ER and PR. Our goal is to explore the role of RUNX2 and its relationship with hormone receptors in BrCa. The aim of this work was to evaluate the effect of endocrine therapy in RUNX2 overexpressing tumors. RUNX2 and control cells (C, empty vector) were injected into the flank of NSG mice. Animals were treated for 3 weeks with an antiestrogen (Fulvestrant, FUL; 0.5 mg/week) or an antiprogestin (Mifepristone, MFP; 6 mg pellets). Control tumors showed a significant growth inhibition with the therapy (C-T47D $p < 0.0001$ C vs FUL and MFP; C-IBH6 $p < 0.0001$ C vs FUL), a lower Ki67 index (C-T47D: $p < 0.0001$ C vs FUL, $p < 0.05$ C vs MFP, C-IBH6 $p < 0.05$ C vs FUL) and higher stromal remodeling compared with untreated ones. In both models, RUNX2 tumors were resistant to endocrine therapy and all animals bearing RUNX2-T47D tumors developed lung metastasis. Our conclusion is that RUNX2 promotes BrCa progression and is a key player in